Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_

**Cellular Respiration Lab**

Ms. Ottolini, AP Biology

**Background**

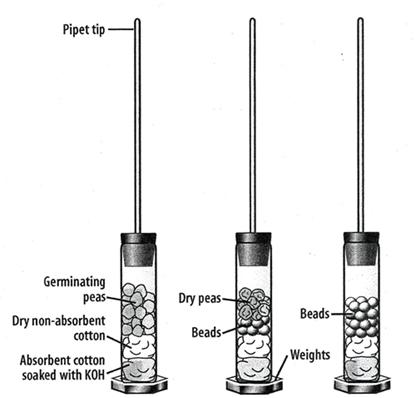
Each individual cell is responsible for the energy exchanges necessary to sustain its ordered structure.  Cells accomplish this task by breaking down nutrient molecules to generate ATP (adenosine triphosphate), which can then be used to run cellular processes that require energy.  This process is called cellular respiration which requires nutrient molecules and oxygen.   Carbon dioxide and water are products of the series of reactions involved in cellular respiration.

equation

There are several methods of indirectly measuring the rate of cellular respiration in organisms.  One method involves monitoring changes in temperature; since the process of respiration is exergonic (produces heat).  Another method is to measure either the oxygen consumption or the carbon dioxide production.  Respirometers are devices that measure these types of gas volume changes, and therefore provide information about the rate of cellular respiration.

During cellular respiration, two gases are changing in volume.  Oxygen gas is being consumed by the respiring cells and carbon dioxide gas is diffusing out of the cells.  The respirometer, therefore, has to be able to deal with two simultaneously changing gas volumes.  This is accomplished by introducing potassium hydroxide into the device.  KOH absorbs carbon dioxide, following this equation

CO2 + 2KOH --> K2CO3 + H2O

Potassium carbonate ( K2CO3 ) is a solid precipitate.  Any CO2 produced is immediately converted from a gas to a solid and is therefore no longer governed by gas laws.  This allows the respirometer to measure only one variable, the ****consumption of oxygen gas by living cells.

One set of three respirometers will be assembled during this lab exercise.  One respirometer will contain germinated (sprouting) beans, one will contain a mix of non-germinating (dry, nonsprouting) beans and plastic beads, and a third will contain only plastic beads.

The purpose of the beads is to ensure that each respirometer is uniform in volume.  The respirometers will also contain a layer of cotton that has been saturated with KOH so that carbon dioxide will be absorbed.   The respirometers will be submerged in a pan of water; water will flow from an area of high pressure to an area of low pressure.  As oxygen is used up by the respiring seeds, the gas pressure inside the respirometer will decrease and the water will flow into the pipet down its pressure gradient.

**Your Hypothesis:**

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**Materials**:   8 germinating bean seeds,  8 dry seeds, plastic beads,  3 respirometer vials, Weights for vials, 3 stoppers,  1 ml graduated pipets, cotton balls, water bath, 100 ml graduated cylinder, masking tape, dropper bottle of 15% KOH

Safety – wear safety goggles.  KOH is caustic, avoid direct skin contact.  If contact occurs, flush affected area with running water for 10 minutes.

**Procedure:**

1.  Label three paper towels as follows:  1, 2, 3. These numbers will correspond to the respirometers of the same numbers.

2.  Fill a graduated cylinder with 20 ml of water.  Count out 8 germinating beans and place them into the graduated cylinder.   Record the total volume of the beans and water in Data Table #1 below.  Subtract the initial 20 ml to determine the total volume of the germinating beans.  Pour out the water from the graduated cylinder and place the 8 germinating beans on paper towel 1.

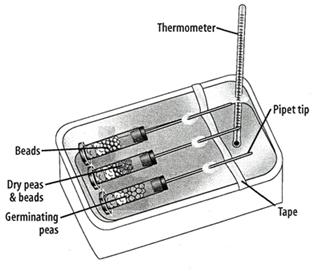
|  |  |  |  |
| --- | --- | --- | --- |
| **DATA TABLE 1: Calculation of Volume in Respirometers** | | | |
|  | Respirometer 1 (germinating beans) | Respirometer 2 (non-germinating beans) | Respirometer 3a (beads only) |
| Initial Volume (mL) | 20 | 20 | 20 |
| Final Volume (mL) |  |  |  |
| Volume of beans/beads |  |  |  |

3.  Fill the graduated cylinder with 20ml of water.  Count out 8 dry (non-germinating) beans and place them into the water.  Drop plastic beads into the cylinder until the final volume is the same as from step 3.

4. Place the bean/bead mixture on paper towel 2.  (Your goal here is to make sure each respirometer has the same volume).

5.  Fill the graduated cylinder with 20 ml of water.  Add beads to the water until the total volume equals the final volume from steps 2 & 3.  Place the beads on paper towel 3.

6.  Assemble the respirometers.  Begin with 3 vials, rubber stoppers and 1 ML pipets.  You will also need masking tape to wrap around the pipet to ensure that there is a tight seal inside the rubber stopper

****7.  Insert the **nontapered** end of one pipet into the upper surface of one of the rubber stoppers.  It should fit tightly.

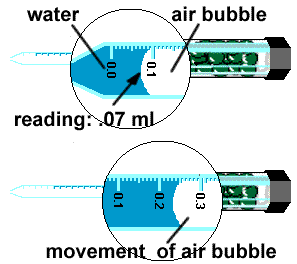
8.  Place half a cotton ball in the bottom of each of the weighted vials.  Push the cotton firmly into the bottom of the vial with the tip of the glass pipet.  Use an eyedropper to drop three drops of 15% KOH on the half cotton ball. Place another half cotton ball on top.

9.  Add the beans, beans/beads, and beads to the appropriate respirometer.  Place the stoppers on each of the vials and ensure they are secured tightly.  Any leaks will cause the experiment to fail.

10.  Place a strip of masking tape across the narrow width of the water bath, approximately 2/3 of the way from one end (see diagram

11.  Place respirometers 1, 2, 3 into water bath so that the pipets rest on the masking tape prop. Leave them like this for five minutes so that the water in the water bath can equilibrate with room temperature.

12. At the end of five minutes, GENTLY submerge each of the tubes entirely in the water bath.  Some water will enter the tip of the pipet, but the influx of water should stop fairly quickly.  If it does not stop, check the respirometer for leaks.

13.  At this point, check to make sure you can read the pipets.

The air bubble should extend from the main chamber up the tube of the pipet.  The pipet may need to be rotated so that you can see the numbers.

14.  If your respirometers float, you may need to weight them with the metal washers.

15.    Record the water level in each pipet onto the data table at the Time Interval 0.

16.  Record the position of the water in each pipet at the end of 5, 10, and 15, 20 min in Data Table 2.

**Methods Summary Chart:**

|  |  |
| --- | --- |
| Independent Variable |  |
| Levels of the Independent Variable (if applicable) |  |
| Dependent Variable |  |
| Method for Measuring Changes in the Dependent Variable |  |
| Control Group (if applicable) |  |
| Constants (factors that stay the same between your control group and your experimental groups) |  |
| Number of Trials |  |

**Data Table: Individual Lab Group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Data Table 2: Calculation of Oxygen Consumption** | | | | | |
| Respirometer 1: Germinating Beans | | | | | |
| Time interval (min): | **0 min** | **5 min** | **10min** | **15 min** | **20 min** |
| Reading, mL |  |  |  |  |  |
| Δ  Volume, mL (reading at 20 mins – reading at time 0) | N/A | N/A | N/A | N/A |  |
| Respirometer 2: Dry Beans | | | | | |
| Reading, mL |  |  |  |  |  |
| Δ  Volume, mL (reading at 20 mins – reading at time 0) | N/A | N/A | N/A | N/A |  |
| Respirometer 3: Beads Only | | | | | |
| Reading, mL |  |  |  |  |  |
| Δ  Volume, mL (reading at 20 mins – reading at time 0) | N/A | N/A | N/A | N/A |  |

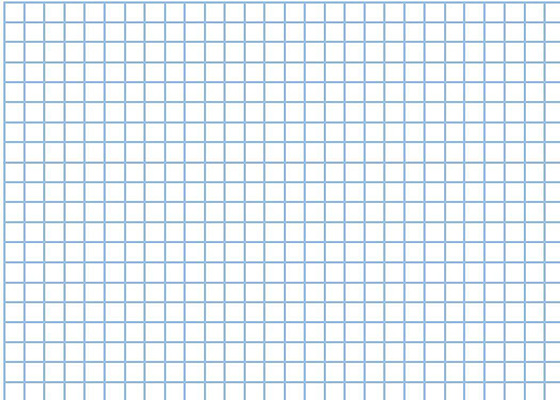
**Data Table: Class Averages (We will draw this data table together after the lab!)**

**Graph:  Rate of Oxygen Consumption in Respirometers (room temperature)**

Plot the independent variable on the X axis (time), and the dependent variable on the Y axis (reading on pipet).

Graph a line for:

1. Germinating beans
2. Dry beans
3. Beads



**Discussion / Conclusion Questions:** Please answer these questions thoroughly and accurately and in complete sentences.

1.   Did your data support your hypothesis? Give specific data to justify your answer.

2.  In this lab exercise, what is the purpose of the ….

1. Beads
2. KOH
3. Respirometer

3.  Explain why the water moved within the pipet.

4. If we had placed the respirometers in cold water instead of room temperature water, how would this have affected the rate of respiration we measured? In other words, would the rate of respiration have increased or decreased, and why?